PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 18 October 1999 (18.10.99)	
International application No. PCT/EP99/00478	**Applicant's or agent's file reference** SCB 468 PCT
International filing date (day/month/year) 27 January 1999 (27.01.99)	**Priority date** (day/month/year) 30 January 1998 (30.01.98)
Applicant MEDICO, Enzo et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

05 August 1999 (05.08.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Nestor Santesso
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

09/600991

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

PCT/EP 99/00478
International Application No.

27 JAN 1999 (27.01.1999)
International Filing Date

EUROPEAN PATENT OFFICE
PCT INTERNATIONAL APPLICATION
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)*: SCB 468 PCT

Box No. I TITLE OF INVENTION

RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

DOMPE' S.p.A.
Via Campo di Pile
67100 L'AQUILA
IT

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MEDICO, Enzo
Via Campo di Pile
67100 L'AQUILA
IT

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MINOJA, Fabrizio
BIANCHETTI BRACCO MINOJA S.r.l.
Via Rossini, 8
20122 MILANO
IT

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☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

See Notes to the request form

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MICHIELI, Paolo
Via Campo di Pile
67100 L'AQUILA
IT

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

COLLESI, Chiara
Via Campo di Pile
67100 L'AQUILA
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This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CASELLI, Gianfranco
Via Calle di Pile
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IT

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

COMOGLIO, Paolo
Via Calle di Pile
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IT

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes: at least one must be marked)*:

Regional Patent

- ☒ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ **AL** Albania .....
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- ☒ **BG** Bulgaria .....
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- ☒ **GM** Gambia
- ☒ **HR** Croatia .....
- ☒ **HU** Hungary .....
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- ☒ **IL** Israel .....
- ☒ **IN** India .....
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- ☒ **KZ** Kazakhstan .....
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- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
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- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg
- ☒ **LV** Latvia
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- ☒ **SK** Slovakia .....
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- ☒ **TM** Turkmenistan .....
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- ☒ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☒ **UZ** Uzbekistan .....
- ☒ **VN** Viet Nam .....
- ☒ **YU** Yugoslavia .....
- ☒ **ZW** Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....
- ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

PCT 99/00478

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 30 JAN 1998 (30.01.1998)	MI98A 000179	IT		
item (2)				
item (3)				

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	:	04
description (excluding sequence listing part)	:	30
claims	:	04
abstract	:	01
drawings	:	19
sequence listing part of description	:	08
Total number of sheets	:	66

This international application is **accompanied by** the item(s) marked below:

1. ☐ fee calculation sheet
2. ☒ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Request for fax acknowledgement

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

January 25, 1999
(25.01.1999)

Fabrizio MINOJA
F Minoja

For receiving Office use only

1. Date of actual receipt of the purported international application: 27 JAN 1999 (27.01.99)
2. Drawings: ☒ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

See Notes to the request form

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/475, 19/00, A61K 38/18 — A3

(11) International Publication Number: **WO 99/38967**

(43) International Publication Date: 5 August 1999 (05.08.99)

(21) International Application Number: PCT/EP99/00478

(22) International Filing Date: 27 January 1999 (27.01.99)

(30) Priority Data:
MI98A000179 30 January 1998 (30.01.98) IT

(71) Applicant *(for all designated States except US)*: DOMPE' S.P.A. [IT/IT]; Via Campo di Pile, I–67100 L'Aquila (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MEDICO, Enzo [IT/IT]; Via Campo di Pile, I–67100 L'Aquila (IT). MICHIELI, Paolo [IT/IT]; Via Campo di Pile, I–67100 L'Aquila (IT). COLLESI, Chiara [IT/IT]; Via Campo di Pile, I–67100 L'Aquila (IT). CASELLI, Gianfranco [IT/IT]; Via Calle di Pile, I–67100 L'Aquila (IT). COMOGLIO, Paolo [IT/IT]; Via Calle di Pile, I–67100 L'Aquila (IT).

(74) Agent: MINOJA, Fabrizio; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I–20122 Milano (IT).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
16 September 1999 (16.09.99)

(54) Title: RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP

(57) Abstract

Recombinant proteins deriving from recombination of structural domains deriving from the α subunits of HGF and/or MSP growth factors. The recombinant proteins of the present invention have biological activity, and protect cells from death (apoptosis) induced by chemotherapeutic drugs. These molecules can conveninently be used to prevent or to treat the toxic side effects of chemotherapeutic agents used in cancer therapy.

INTERNATIONAL SEARCH REPORT

Ir ational Application No
PCT/EP 99/00478

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/475 C07K19/00 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	TRUSOLINO L. ET AL.: "Interactions between scatter factors and their receptors: hints for therapeutic applications" THE FASEB JOURNAL, vol. 12, no. 13, October 1998 (1998-10), pages 1267-1280, XP002109039 page 1274, right-hand column, paragraph 5 - page 1275, left-hand column, paragraph 1 ---	1,2
A	WO 93 23550 A (GENENTECH INC. (US); GODOWSKI PAUL J. (US)) 25 November 1993 (1993-11-25) abstract page 5, line 25 - page 9, line 3; figure 1 page 12, line 5 - page 15, line 26 --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 1999	27/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Macchia, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/EP 99/00478

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 23541 A (GENENTECH INC (US); GODOWSKI PAUL J; LOKKER NATHALIE A; MARK MELANIE R) 25 November 1993 (1993-11-25) abstract page 2, line 28-34 page 7, line 5-24; figure 1 ---	
A	HARTMANN G. ET AL.: "A functional domain in the heavy chain of Scatter Factor / Hepatocyte Growth Factor binds the c-Met receptor and induces cell dissociation but not mitogenesis" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 89, 1 December 1992 (1992-12-01), pages 11574-11578, XP000764839 ISSN: 0027-8424 cited in the application abstract ---	
A	GAUDINO G. ET AL.: "Ron is a heterodimeric tyrosine kinase receptor activated by the HGF homologue MSP" EMBO JOURNAL, vol. 13, no. 15, 1 January 1994 (1994-01-01), pages 3524-3532, XP002036128 ISSN: 0261-4189 cited in the application page 3528, left-hand column, paragraph 2 ---	
A	WO 94 06456 A (GENENTECH INC. (US); ROOS FILIP (US); SCHWALL RALPH (US)) 31 March 1994 (1994-03-31) abstract -----	12,13

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/EP 99/00478

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9323550 A	25-11-1993	US 5316921 A	31-05-1994
		US 5328837 A	12-07-1994
		EP 0642580 A	15-03-1995
		EP 0642585 A	15-03-1995
		JP 7508420 T	21-09-1995
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		US 5547856 A	20-08-1996
		WO 9323541 A	25-11-1993
		US 5580963 A	03-12-1996
		US 5684136 A	04-11-1997
		US 5763584 A	09-06-1998
		US 5770704 A	23-06-1998
WO 9323541 A	25-11-1993	US 5316921 A	31-05-1994
		US 5328837 A	12-07-1994
		EP 0642580 A	15-03-1995
		EP 0642585 A	15-03-1995
		JP 7508420 T	21-09-1995
		JP 7508178 T	14-09-1995
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		WO 9323550 A	25-11-1993
		US 5580963 A	03-12-1996
		US 5684136 A	04-11-1997
		US 5763584 A	09-06-1998
		US 5770704 A	23-06-1998
WO 9406456 A	31-03-1994	CA 2144081 A	31-03-1994
		DE 69310525 D	12-06-1997
		DE 69310525 T	02-10-1997
		EP 0661993 A	12-07-1995
		JP 8501314 T	13-02-1996
		US 5654404 A	05-08-1997
		US 5703048 A	30-12-1997

CLAIMS

1. Recombinant proteins comprising two superdomains, separated by a spacer sequence (linker), obtained combining the HL and K1-K4 domains of HGF and MSP α chains.

2. Recombinant proteins as claimed in claim 1, of general formula (I):

[A] - B - [C] - (D)y (I)

in which

[A] corresponds to the sequence $(LS)_m$-HL-K1-$(K2)_n$-$(K3)_o$-$(K4)_p$

wherein (the numbering of the following amino acids refers to the HGF and MSP sequences as reported in Fig. 1 and 2, respectively):

LS is an amino acid sequence corresponding to residues 1-31 of HGF or 1-18 of MSP;

HL is an amino acid sequence derived from the α chain of HGF starting between residues 32-70 and ending between residues 96-127; or it is an amino acid sequence derived from the α chain of MSP starting between residues 19-56 and ending between residues 78-109;

K1 is an amino acid sequence derived from the α chain of HGF starting between residues 97-128 and ending between residues 201-205; or it is an amino acid sequence derived from the α chain of MSP starting between residues 79-110 and ending between residues 186-190;

K2 is an amino acid sequence derived from the α chain of HGF starting between residues 202-206 and ending between residues 283-299; or it is an amino acid sequence derived from the α chain of MSP starting between residues 187-191 and ending between residues 268-282;

K3 is an amino acid sequence derived from the α chain of HGF starting between residues 284-300 and ending between residues 378-385; or it is an amino acid sequence derived from the α chain of MSP starting between residues 269-283 and ending between residues 361-369;

K4 is an amino acid sequence derived from the α chain of HGF starting between residues 379-386 and ending between residues 464-487; or it is an amino acid sequence derived from the α chain of MSP starting between residues 362-370 and ending between residues 448-481;

m, n, o, p are 0 or 1;

the sum n + o + p is an integer from 1 to 3 or 0, with the proviso that $n \geq o \geq p$;

B is the sequence $[(X)_q\ Y]_r$, wherein X = Gly and Y = Ser, or Cys, or Met, or Ala;

q is an integer from 2 to 8;

r is an integer from 1 to 9;

[C] corresponds to the sequence HL-K1-$(K2)_s$-$(K3)_t$-$(K4)_u$

wherein HL, K1-K4 are as defined above,

s, t, u are 0 or 1; the sum s + t + u is an integer from 1 to 3 or 0, with the proviso that $s \geq t \geq u$;

D is the sequence W-Z, wherein W is a conventional proteolytic site, Z is any tag sequence useful for the purification and detection of the protein; y is 0 or 1.

3. Recombinant proteins according to claims 1-2, in which the HL domain is a sequence of HGF α chain ranging from amino acids 32 to 127, or a sequence of MPS α chain ranging from amino acids 19 to 98; the K1

domain is a sequence of HGF α chain ranging from amino acids 128 to 203, or a sequence of MPS α chain ranging from amino acids 99 to 188; the K2 domain is a sequence of HGF α chain ranging from amino acids 204 to 294, or a sequence of MPS α chain ranging from amino acids 189 to 274; the K3 domain is a sequence of HGF α chain ranging from amino acids 286 to 383, or a sequence of MPS α chain ranging from amino acids 275 to 367; the K4 domain is a sequence of HGF α chain ranging from amino acids 384 to 487, or a sequence of MPS α chain ranging from amino acids 368 to 477.

4. Recombinant proteins according to claims 1-3 of formula (II):

LS_{MSP}-HL_{MSP}-$K1_{MSP}$-$K2_{MSP}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (II)

in which LS_{MSP} is the sequence 1-18 of MSP, HL_{MSP} is the sequence 19-56 of MSP, $K1_{MSP}$ is the sequence 99-188 of MSP, $K2_{MSP}$ is the sequence 189-274 of MSP, HL_{HGF} is the sequence 32-127 of HGF, $K1_{HGF}$ is the sequence 128-203 of HGF, $K2_{HGF}$ is the sequence 204-294 of HGF, L is the sequence $(Gly_4Ser)_3$, D is the sequence Asp_4-Lys-His_6.

5. Recombinant proteins according to claims 1-3 of formula (III):

LS_{HGF}-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (III)

in which HL_{HGF}, $K1_{HGF}$, $K2_{HGF}$, L and D are as defined in claim 4, LS_{HGF} is the sequence 1-31 of HGF.

6. Nucleotide sequences encoding for the recombinant proteins of claims 1-5.

7. Expression vectors comprising the nucleotide sequences of claim 6.

8. Prokaryotic or eukaryotic host cell transformed with the expression vector of claim 7.

9. Process for preparing the recombinant proteins of claims 1-5, which comprises the following steps:

a) construction of DNA encoding the desired protein;

b) insertion of DNA in an expression vector;

c) transformation of a host cell with recombinant DNA (rDNA);

d) culture of the transformed host cell so as to express the recombinant protein;

e) extraction and purification of the produced recombinant protein.

10. Process according to claim 9, wherein the host cell is kidney epithelial BOSC cell or SF9 insect cell.

11. Recombinant proteins of claims 1-5 for use as therapeutical agents.

12. Use of recombinant proteins of claims 1-5 in the manufacture of a medicament for the prevention or treatment of chemotherapeutic-induced toxicity.

13. Use according to claim 10, wherein the chemotherapeutic-induced toxicity is myelotoxicity, kidney toxicity, neurotoxicity, mucotoxicity and hepatotoxicity.

14. Pharmaceutical compositions containing an effective amount of the recombinant proteins of claims 1-5, in combination with pharmacologically acceptable excipients.

09/600991

16

PATENT COOPERATION TREATY

REC'D 17 APR 2000

WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB468 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/00478	27/01/1999	30/01/1998
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant DOMPE' S.p.A. et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/08/1999	11.04.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Perez, C Telephone No. +49 89 2399 2484

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/00478

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-30	as originally filed				

Claims, No.:

1-13	as received on	13/03/2000	with letter of	13/03/2000	

Drawings, sheets:

1/19-19/19	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has

been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 10-12 with regard to industrial appl.

because:

☒ the said international application, or the said claims Nos. 10-12 with regard to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-13
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-9 and 13
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Additional remarks to item I (basis of opinion)

- since all the sequences of the second Listing received the 03.03.1999 (SEQ ID N°1 to 16) were filed with the present application, said listing is considered to meet the to requirements of Article 34(2b) and Rule 70.2 (c) PCT.
- the amended set of claims filed with the letter dated 13.03.2000 fulfill the requirements of Article 34 (2b) and Rule 70.2 (c) PCT.

Thus, the present international preliminary examination report is based on the amended set of claims.

2. Additional remark to item II (priority, Article 8 PCT)

The right of priority covers only those elements included in the priority document (Article 8 PCT). Actually, the IPEA considers that at least a part of the subject-matter disclosed within the present application does not seem to have a basis in the application whose priority is claimed: for example, claims 8-9 as well as the subject-matter described within examples 1c, 4b, 5 and 6 and figure 8.

3. Additional remark to item III (no opinion)

Claims 10-12 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT (medical use). Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

4. Additional remarks to item V (reasoned statement under Rule 66.2(a) (ii) with regard to novelty, inventive step or industrial applicability)

4.1 Present application

The present application discloses recombinant proteins comprising two structural domains obtained by combination of the hairpin loop (HL) and kringle (K1 to K4) domains of hepatocyte growth factor (HGF) and/or macrophage stimulating protein (MSP) α subunits, which are linked together by a spacer sequence (linker). In particular, the engineered factors comprise two domains ([A] and [C]) separated by a spacer (B), each of them comprising at least an hairpin loop (HL) and the kringle 1 domain (K1) of HGF and/or MSP (see formula I). Two recombinant proteins are exemplified:

- Metron Factor 1 (Metron F-1) comprises sequence 1-56 and 99-274 of MSP linked with the sequence $(Gly_4Ser)_3$ to sequence 32-294 of HGF, followed by the tag

sequence Asp_4-Lys-His_6.
- Magic Factor 1 (Magic F-1) consists of sequence 1-294 of HGF linked with the sequence $(Gly_4Ser)_3$ to sequence 19-274 of MSP, followed by the tag sequence Asp_4-Lys-His_6.

Also disclosed are nucleotides sequences encoding said proteins, expression vectors comprising said nucleotides, host cells transformed by said vectors, and process for preparing said recombinant proteins, using for example kidney BOSC cell or SF9 insect cell. Finally, the application discloses said proteins for use as therapeutical agents, their use in the manufacture of a medicament for the prevention or treatment of chemotherapeutic-induced toxicity and pharmaceutical compositions containing an effective amount of said proteins combined with a pharmacologically acceptable excipient.

4.2 Prior art documents

The following documents are considered to be relevant for assessing the novelty and inventiveness of the claimed subject-matter:

D1: WO-A-9323550

D2: Embo J., vol. 13, n°15, 1994, Gaudino et al., p. 3524-3532

D3: Embo J., vol. 11, n°7, 1992, Lokker et al., p. 2503-2510

i) **D1** describes conjugates of two ligands (hetero dimers) capable of binding to tyrosine kinase receptors, the two ligands being fused by a linker, and the conjugate triggering receptor activation (D1: p.78, claims 13 and 15). In particular, it also discloses HGF-IgG dimers, such as NK2 HGF-IgG, comprising the NK2 deletion variant of HGF fused with an IgG-γ1 heavy chain, thereby producing chimeras which are expressed as dimers (D1: p.65, l.1 - p.66, l.4). Each superdomain (NK2 HGF) comprises HL, K1 and K2 domains of HGF α chain (NK2 deletion mutant) (D1: p.23, l.4-10 and p.65, l.1-21). Hence, the biologically inactive HGF variant NK2 HGF recovers a wild-type HGF biological activity when binding to the receptor as an HGF variant-IgG dimeric chimera (D1: p.6, l.24-28). Moreover, said recombinant protein was shown to bind to HGF receptor similarly to wild-type ligand (D1: p.66, l.19-21) and to exhibit mitogenic activity on hepatocytes culture (D1: p.67, l.1-9). Also described are nucleotide sequence, expression vector, cell producing NK2 HGF-IgG, and a process for preparing recombinant NK2 HGF-IgG (D1: p.65, l.1 - p.66, l.21).

ii) **D2** discloses a recombinant fusion protein, MSP-NK2 , which comprises the N-terminal portion of MSP α chain including the two first kringles (HL, K1 and K2) linked to a fragment of IgG1-γ1 heavy chain and which is capable of stimulating phosphorylation of MSP receptor (Ron) (D2: p.3528, col.1, l.19-31).

4.3 Statement with regard to novelty (Article 33(2) PCT)

The subject-matter of claims 1-13 meets the requirements of Article 33 (2) PCT in view of the available prior art documents.

Actually, the fusion protein of D2 comprises only one domain of MSP (see § 4.2 ii). Moreover, the claimed recombinant proteins differ from the HGF-IgG dimers of D1, by the nature of the linker (the sequence B versus IgG-γ1 heavy chain in D1) (see § 4.2 i). Moreover, the claimed recombinant proteins result from the asymmetrical linking of the superdomains A et B to the spacer (asymmetrical tandem array), whereas the dimer of D1 result from the binding of the linker to identical site of HGF (symmetrical dimer).

4.4 Statement with regard to inventive step (Article 33(3) PCT)

The subject-matter of claims 1-13 fulfill the requirements of Article 33(3) PCT, because said claims do involve an inventive step in view of the available prior art documents.

Neither D1, nor D2 suggests to combine HL and K domains according to formula 1 in order to recover the desired activities of HGF or MSP cytokines such as promotion of scattering of hematopoietic precursors cells and protecting activity against antineoplastic treatment-induced apoptosis of liver, kidney and gastroenteric cells, without their unfavourable effects such as mitogenic activity on neoplastic cell (see § 4.2). Therefore, claims 1-13 meet the requirements of Article 33(3) PCT in view of the available prior art documents.

4.5 Statement with regard to industrial applicability (Article 33(4) PCT)

For the assessment of the present claims 10-12 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The attention of the applicant is also drawn to the fact, that the patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not

recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

5. Additional remarks to item VIII (certain observations on the international application, Article 6 PCT)

5.1 Claim 1

Claim 1 does not fulfill the requirements of Article 6 PCT, because its subject-matter is not clearly defined:

- it is not clear, from the wording of said claim, how HL and K1-K4 domains of HGF and/or MSP α chains should be combined to obtain the claimed recombinant proteins. For example, it is not clear whether one superdomain only comprises a combination of said HGF domains (HL, and K1-K4 domains), and the other superdomain a combination of said MSP domains, or if each superdomain may comprise a mixture of said domains of both ligands. The attention of the applicant is drawn to the fact, that each superdomain of the two exemplified proteins, Magic and Metron factors, corresponds to the combination of different domains of the same factor (the HL and K1-K4 domains comprised in one superdomain are all derived from the same ligand), but not to the mixture of domains of HGF and MSP domains.
- the component D of the recombinant protein disclosed in claim 2 is not clearly defined, because its component Z is defined in terms of the result to be achieved ("useful for the purification and detection of ..."). Such a definition is generally not allowable, because it merely amounts to a statement of the underlying problem (see PCT Gazette, 29.10.1998, "Guidelines concerning PCT international preliminary examination", Section IV, Chapter III-4.7).
- the term "conventional" used to define the proteolytic site W is vague, and, as such, renders the scope of said claim unclear.

5.2 Additional comments

New claim 2 as well as **claim 5**, refer back, among other, to themselves. This renders the scope of said claims unclear.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/00478

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/475 C07K19/00 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	TRUSOLINO L. ET AL.: "Interactions between scatter factors and their receptors: hints for therapeutic applications" THE FASEB JOURNAL, vol. 12, no. 13, October 1998 (1998-10), pages 1267-1280, XP002109039 page 1274, right-hand column, paragraph 5 - page 1275, left-hand column, paragraph 1 ---	1,2
A	WO 93 23550 A (GENENTECH INC. (US); GODOWSKI PAUL J. (US)) 25 November 1993 (1993-11-25) abstract page 5, line 25 - page 9, line 3; figure 1 page 12, line 5 - page 15, line 26 --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 1999	27/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Macchia, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/00478

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 23541 A (GENENTECH INC (US); GODOWSKI PAUL J; LOKKER NATHALIE A; MARK MELANIE R) 25 November 1993 (1993-11-25) abstract page 2, line 28-34 page 7, line 5-24; figure 1	
A	HARTMANN G. ET AL.: "A functional domain in the heavy chain of Scatter Factor / Hepatocyte Growth Factor binds the c-Met receptor and induces cell dissociation but not mitogenesis" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 89, 1 December 1992 (1992-12-01), pages 11574-11578, XP000764839 ISSN: 0027-8424 cited in the application abstract	
A	GAUDINO G. ET AL.: "Ron is a heterodimeric tyrosine kinase receptor activated by the HGF homologue MSP" EMBO JOURNAL, vol. 13, no. 15, 1 January 1994 (1994-01-01), pages 3524-3532, XP002036128 ISSN: 0261-4189 cited in the application page 3528, left-hand column, paragraph 2	
A	WO 94 06456 A (GENENTECH INC. (US); ROOS FILIP (US); SCHWALL RALPH (US)) 31 March 1994 (1994-03-31) abstract	12,13

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/00478

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9323550 A	25-11-1993	US 5316921 A	31-05-1994
		US 5328837 A	12-07-1994
		EP 0642580 A	15-03-1995
		EP 0642585 A	15-03-1995
		JP 7508420 T	21-09-1995
		JP 7508178 T	14-09-1995
		US 5547856 A	20-08-1996
		WO 9323541 A	25-11-1993
		US 5580963 A	03-12-1996
		US 5684136 A	04-11-1997
		US 5763584 A	09-06-1998
		US 5770704 A	23-06-1998
WO 9323541 A	25-11-1993	US 5316921 A	31-05-1994
		US 5328837 A	12-07-1994
		EP 0642580 A	15-03-1995
		EP 0642585 A	15-03-1995
		JP 7508420 T	21-09-1995
		JP 7508178 T	14-09-1995
		US 5547856 A	20-08-1996
		WO 9323550 A	25-11-1993
		US 5580963 A	03-12-1996
		US 5684136 A	04-11-1997
		US 5763584 A	09-06-1998
		US 5770704 A	23-06-1998
WO 9406456 A	31-03-1994	CA 2144081 A	31-03-1994
		DE 69310525 D	12-06-1997
		DE 69310525 T	02-10-1997
		EP 0661993 A	12-07-1995
		JP 8501314 T	13-02-1996
		US 5654404 A	05-08-1997
		US 5703048 A	30-12-1997

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
SCB468 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 00478	27/01/1999	30/01/1998
Applicant		
DOM'S.P.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of Invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/00478

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

Recombinant proteins deriving from recombination of structural domains deriving from the alpha subunits of HGF and/or MSP growth factors. The recombinant proteins of the present invention have biological activity, and protect cells from death (apoptosis) induced by chemotherapeutic drugs. These molecules can be conveniently used to prevent or to treat the toxic side effects of chemotherapeutic agents used in cancer therapy.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB468 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/00478	27/01/1999	30/01/1998
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant DOMPE' S.p.A. et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/08/1999	11.04.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax +49 89 2399 - 4465	Authorized officer Perez, C Telephone No. +49 89 2399 2484

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/00478

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-30	as originally filed				

Claims, No.:

1-13	as received on	13/03/2000	with letter of	13/03/2000	

Drawings, sheets:

1/19-19/19	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has

been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 10-12 with regard to industrial appl.

because:

☒ the said international application, or the said claims Nos. 10-12 with regard to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-13
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-9 and 13
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. **Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. **Additional remarks to item I (basis of opinion)**

- since all the sequences of the second Listing received the 03.03.1999 (SEQ ID N°1 to 16) were filed with the present application, said listing is considered to meet the to requirements of Article 34(2b) and Rule 70.2 (c) PCT.
- the amended set of claims filed with the letter dated 13.03.2000 fulfill the requirements of Article 34 (2b) and Rule 70.2(c) PCT.

Thus, the present international preliminary examination report is based on the amended set of claims.

2. **Additional remark to item II (priority, Article 8 PCT)**

The right of priority covers only those elements included in the priority document (Article 8 PCT). Actually, the IPEA considers that at least a part of the subject-matter disclosed within the present application does not seem to have a basis in the application whose priority is claimed: for example, claims 8-9 as well as the subject-matter described within examples 1c, 4b, 5 and 6 and figure 8.

3. **Additional remark to item III (no opinion)**

Claims 10-12 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT (medical use). Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

4. **Additional remarks to item V (reasoned statement under Rule 66.2(a) (ii) with regard to novelty, inventive step or industrial applicability)**

4.1 **Present application**

The present application discloses recombinant proteins comprising two structural domains obtained by combination of the hairpin loop (HL) and kringle (K1 to K4) domains of hepatocyte growth factor (HGF) and/or macrophage stimulating protein (MSP) α subunits, which are linked together by a spacer sequence (linker). In particular, the engineered factors comprise two domains ([A] and [C]) separated by a spacer (B), each of them comprising at least an hairpin loop (HL) and the kringle 1 domain (K1) of HGF and/or MSP (see formula I). Two recombinant proteins are exemplified:

- Metron Factor 1 (Metron F-1) comprises sequence 1-56 and 99-274 of MSP linked with the sequence $(Gly_4Ser)_3$ to sequence 32-294 of HGF, followed by the tag

sequence Asp_4-Lys-His_6.

- Magic Factor 1 (Magic F-1) consists of sequence 1-294 of HGF linked with the sequence $(Gly_4Ser)_3$ to sequence 19-274 of MSP, followed by the tag sequence Asp_4-Lys-His_6.

Also disclosed are nucleotides sequences encoding said proteins, expression vectors comprising said nucleotides, host cells transformed by said vectors, and process for preparing said recombinant proteins, using for example kidney BOSC cell or SF9 insect cell. Finally, the application discloses said proteins for use as therapeutical agents, their use in the manufacture of a medicament for the prevention or treatment of chemotherapeutic-induced toxicity and pharmaceutical compositions containing an effective amount of said proteins combined with a pharmacologically acceptable excipient.

4.2 Prior art documents

The following documents are considered to be relevant for assessing the novelty and inventiveness of the claimed subject-matter:

D1: WO-A-9323550

D2: Embo J., vol. 13, n°15, 1994, Gaudino et al., p. 3524-3532

D3: Embo J., vol. 11, n°7, 1992, Lokker et al., p. 2503-2510

i) **D1** describes conjugates of two ligands (hetero dimers) capable of binding to tyrosine kinase receptors, the two ligands being fused by a linker, and the conjugate triggering receptor activation (D1: p.78, claims 13 and 15). In particular, it also discloses HGF-IgG dimers, such as NK2 HGF-IgG, comprising the NK2 deletion variant of HGF fused with an IgG-γ1 heavy chain, thereby producing chimeras which are expressed as dimers (D1: p.65, l.1 - p.66, l.4). Each superdomain (NK2 HGF) comprises HL, K1 and K2 domains of HGF α chain (NK2 deletion mutant) (D1: p.23, l.4-10 and p.65, l.1-21). Hence, the biologically inactive HGF variant NK2 HGF recovers a wild-type HGF biological activity when binding to the receptor as an HGF variant-IgG dimeric chimera (D1: p.6, l.24-28). Moreover, said recombinant protein was shown to bind to HGF receptor similarly to wild-type ligand (D1: p.66, l.19-21) and to exhibit mitogenic activity on hepatocytes culture (D1: p.67, l.1-9). Also described are nucleotide sequence, expression vector, cell producing NK2 HGF-IgG, and a process for preparing recombinant NK2 HGF-IgG (D1: p.65, l.1 - p.66, l.21).

ii) D2 discloses a recombinant fusion protein, MSP-NK2 , which comprises the N-terminal portion of MSP α chain including the two first kringles (HL, K1 and K2) linked to a fragment of IgG1-γ1 heavy chain and which is capable of stimulating phosphorylation of MSP receptor (Ron) (D2: p.3528, col.1, l.19-31).

4.3 Statement with regard to novelty (Article 33(2) PCT)

The subject-matter of claims 1-13 meets the requirements of Article 33 (2) PCT in view of the available prior art documents.

Actually, the fusion protein of D2 comprises only one domain of MSP (see § 4.2 ii). Moreover, the claimed recombinant proteins differ from the HGF-IgG dimers of D1, by the nature of the linker (the sequence B versus IgG-γ1 heavy chain in D1) (see § 4.2 i). Moreover, the claimed recombinant proteins result from the asymmetrical linking of the superdomains A et B to the spacer (asymmetrical tandem array), whereas the dimer of D1 result from the binding of the linker to identical site of HGF (symmetrical dimer).

4.4 Statement with regard to inventive step (Article 33(3) PCT)

The subject-matter of claims 1-13 fulfill the requirements of Article 33(3) PCT, because said claims do involve an inventive step in view of the available prior art documents.

Neither D1, nor D2 suggests to combine HL and K domains according to formula 1 in order to recover the desired activities of HGF or MSP cytokines such as promotion of scattering of hematopoietic precursors cells and protecting activity against antineoplastic treatment-induced apoptosis of liver, kidney and gastroenteric cells, without their unfavourable effects such as mitogenic activity on neoplastic cell (see § 4.2). Therefore, claims 1-13 meet the requirements of Article 33(3) PCT in view of the available prior art documents.

4.5 Statement with regard to industrial applicability (Article 33(4) PCT)

For the assessment of the present claims 10-12 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The attention of the applicant is also drawn to the fact, that the patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not

recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

5. Additional remarks to item VIII (certain observations on the international application, Article 6 PCT)

5.1 Claim 1

Claim 1 does not fulfill the requirements of Article 6 PCT, because its subject-matter is not clearly defined:

- it is not clear, from the wording of said claim, how HL and K1-K4 domains of HGF and/or MSP α chains should be combined to obtain the claimed recombinant proteins. For example, it is not clear whether one superdomain only comprises a combination of said HGF domains (HL, and K1-K4 domains), and the other superdomain a combination of said MSP domains, or if each superdomain may comprise a mixture of said domains of both ligands. The attention of the applicant is drawn to the fact, that each superdomain of the two exemplified proteins, Magic and Metron factors, corresponds to the combination of different domains of the same factor (the HL and K1-K4 domains comprised in one superdomain are all derived from the same ligand), but not to the mixture of domains of HGF and MSP domains.
- the component D of the recombinant protein disclosed in claim 2 is not clearly defined, because its component Z is defined in terms of the result to be achieved ("useful for the purification and detection of ..."). Such a definition is generally not allowable, because it merely amounts to a statement of the underlying problem (see PCT Gazette, 29.10.1998, "Guidelines concerning PCT international preliminary examination", Section IV, Chapter III-4.7).
- the term "conventional" used to define the proteolytic site W is vague, and, as such, renders the scope of said claim unclear.

5.2 Additional comments

New claim 2 as well as **claim 5**, refer back, among other, to themselves. This renders the scope of said claims unclear.

CLAIMS

1. Recombinant proteins comprising two superdomains, separated by a spacer sequence (linker), obtained combining the HL and K1-K4 domains of HGF and/or MSP α chains, according to general formula (I):

$$[A] - B - [C] - (D)y \qquad (I)$$

in which

[A] corresponds to the sequence $(LS)_m$-HL-K1-$(K2)_n$-$(K3)_o$-$(K4)_p$

wherein (the numbering of the following amino acids refers to the HGF and MSP sequences as reported in Fig. 1 and 2, respectively):

LS is an amino acid sequence corresponding to residues 1-31 of HGF or 1-18 of MSP;

HL is an amino acid sequence starting between residues 32-70 of HGF α chain and ending between residues 96-127 of the identical chain; or it is an amino acid sequence starting between residues 19-56 of MSP α chain and ending between residues 78-109 of the identical chain;

K1 is an amino acid sequence starting between residues 97-128 of HGF α chain and ending between residues 201-205 of the identical chain; or it is an amino acid sequence starting between residues 79-110 of MSP α chain and ending between residues 186-190 of the identical chain;

K2 is an amino acid sequence starting between residues 202-206 of HGF α chain and ending between residues 283-299 of the identical chain; or it is an amino acid sequence starting between residues 187-191 of MSP α chain and ending between residues 268-282 of the identical chain;

K3 is an amino acid sequence starting between residues 284-300 of HGF α chain and ending between residues 378-385 of the identical chain; or it is

an amino acid sequence starting between residues 269-283 of MSP α chain and ending between residues 361-369 of the identical chain;

K4 is an amino acid sequence starting between residues 379-386 of HGF α chain and ending between residues 464-487 of the identical chain; or it is an amino acid sequence starting between residues 362-370 of MSP α chain and ending between residues 448-481 of the identical chain;

m, n, o, p are 0 or 1;

the sum n + o + p is an integer from 1 to 3 or 0, with the proviso that n ≥ o ≥ p;

B is the sequence $[(X)_q\ Y]_r$, wherein X = Gly and Y = Ser, or Cys, or Met, or Ala;

q is an integer from 2 to 8;

r is an integer from 1 to 9;

[C] corresponds to the sequence HL-K1-$(K2)_s$-$(K3)_t$-$(K4)_u$

wherein HL, K1-K4 are as defined above,

s, t, u are 0 or 1; the sum s + t + u is an integer from 1 to 3 or 0, with the proviso that s ≥ t ≥ u;

D is the sequence W-Z, wherein W is a conventional proteolytic site, Z is any tag sequence useful for the purification and detection of the protein; y is 0 or 1.

2. Recombinant proteins according to claims 1-2, in which the HL domain is a sequence of HGF α chain ranging from amino acids 32 to 127, or a sequence of MPS α chain ranging from amino acids 19 to 98; the K1 domain is a sequence of HGF α chain ranging from amino acids 128 to 203, or a sequence of MPS α chain ranging from amino acids 99 to 188; the K2 domain is a sequence of HGF α chain ranging from amino acids 204 to 294,

or a sequence of MPS α chain ranging from amino acids 189 to 274; the K3 domain is a sequence of HGF α chain ranging from amino acids 286 to 383, or a sequence of MPS α chain ranging from amino acids 275 to 367; the K4 domain is a sequence of HGF α chain ranging from amino acids 384 to 487, or a sequence of MPS α chain ranging from amino acids 368 to 477.

3. Recombinant proteins according to claims 1-2 of formula (II):

LS_{MSP}-HL_{MSP}-$K1_{MSP}$-$K2_{MSP}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (II)

in which LS_{MSP} is the sequence 1-18 of MSP, HL_{MSP} is the sequence 19-56 of MSP, $K1_{MSP}$ is the sequence 99-188 of MSP, $K2_{MSP}$ is the sequence 189-274 of MSP, HL_{HGF} is the sequence 32-127 of HGF, $K1_{HGF}$ is the sequence 128-203 of HGF, $K2_{HGF}$ is the sequence 204-294 of HGF, L is the sequence $(Gly_4Ser)_3$, D is the sequence Asp_4-Lys-His_6.

4. Recombinant proteins according to claims 1-2 of formula (III):

LS_{HGF}-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (III)

in which HL_{HGF}, $K1_{HGF}$, $K2_{HGF}$, L and D are as defined in claim 4, LS_{HGF} is the sequence 1-31 of HGF.

5. Nucleotide sequences encoding for the recombinant proteins of claims 1-5.

6. Expression vectors comprising the nucleotide sequences of claim 5.

7. Prokaryotic or eukaryotic host cell transformed with the expression vector of claim 6.

8. Process for preparing the recombinant proteins of claims 1-4, which comprises the following steps:

a) construction of DNA encoding the desired protein;

b) insertion of DNA in an expression vector;

c) transformation of a host cell with recombinant DNA (rDNA);

d) culture of the transformed host cell so as to express the recombinant protein;

e) extraction and purification of the produced recombinant protein.

9. Process according to claim 8, wherein the host cell is kidney epithelial BOSC cell or SF9 insect cell.

10. Recombinant proteins of claims 1-4 for use as therapeutic agents.

11. Use of recombinant proteins of claims 1-4 in the manufacture of a medicament for the prevention or treatment of chemotherapeutic-induced toxicity.

12. Use according to claim 9, wherein the chemotherapeutic-induced toxicity is myelotoxicity, kidney toxicity, neurotoxicity, mucotoxicity and hepatotoxicity.

13. Pharmaceutical compositions containing an effective amount of the recombinant proteins of claims 1-4, in combination with pharmacologically acceptable excipients.